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Imbalanced coral growth between organic tissue and carbonate skeleton caused by nutrient enrichment

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Abstract

Effects of moderate nutrient enrichment (NO_3^-: <5 μmol L^{-1}, PO_4^{3-}: <0.3 μmol L^{-1}) on two carbon (C) fixation rates (photosynthesis and calcification) of the zooxanthellate coral *Acropora pulchra* were investigated under laboratory conditions. The coral branches were incubated in the nutrient condition for three different periods (0, 5, 10 d) to observe changes in tissue biomass and zooxanthellate chlorophyll *a* (Chl *a*) concentration. Next, the incubated corals were simultaneously transferred to nutrient-depleted seawater containing ^{13}C-labeled dissolved inorganic carbon to assay net photosynthesis and calcification rates. Chl *a* concentration per unit surface area increased 2.6-fold for the 10-d enrichment, and net photosynthetic rates were also stimulated up to a similar level (2.8-fold). Tissue biomass of the host coral and zooxanthellae was approximately doubled during the period. On the other hand, calcification rates only increased 1.3-fold, suggesting that even moderate nutrient loading resulted in one-sided enhancement of the algal photosynthetic activity. The measured C fixation ratios of organic C : skeletal C were higher than the structural ratios, and the inconsistency became greater as Chl *a* concentration increased. The increased photosynthetic products could be excessively stored in the organic tissue and/or released into the ambient seawater.

Hermatypic corals play a significant role for carbon (C) dynamics in tropical and subtropical coastal seawater through calcification by the animal coral and photosynthesis by its symbiotic algae (zooxanthellae). These C fixation processes strongly influence carbonate chemistry in reef waters and thus CO_2 exchange with the atmosphere. Because calcification potentially releases gaseous CO_2 (Ware et al. 1991), whether atmospheric CO_2 is absorbed or released from coral reefs depends on the proportion of photosynthesis to calcification.

It has long been considered that coral calcification and algal photosynthesis are strongly coupled to each other. Calcification rates have been observed to be higher in light than in dark (e.g., Kawaguti and Sakumoto 1948), suggesting photosynthetic stimulation of calcification. The opposite interaction, i.e., stimulation of photosynthesis by calcification, has also been suggested in several studies (McConnaughey 1991; McConnaughey and Whelan 1997), though Gattuso et al. (2000) argued that CO_2 released by calcification was not a significant source for algal photosynthesis. Suzuki et al. (1995) simulated a model of coexisting effects of photosynthesis and calcification and showed that both reactions potentially enhance each other.

Currently, increase in nutrient concentration has been observed in many reefs and the effect on reef organisms is increasingly becoming an issue (Walker and Ormond 1982; Tomascik and Sander 1985). For hermatypic coral colonies, dissolved inorganic nutrients potentially enhance zooxanthellate photosynthetic activity (Dubinsky et al. 1990; Marubini and Davies 1996; Ferrier-Pagès et al. 2000*a*). Both increases in algal population density per unit surface area of the coral and Chl *a* concentration per algal cell have been observed in previous studies (Hoegh-Guldberg and Smith 1989; Muller-Parker et al. 1994; Marubini and Davies 1996). Coral and algal tissue biomass has also been reported to increase (Muller-Parker et al. 1994).

Many studies have shown negative effects of nutrient enrichment on bulk coral growth (Stambler et al. 1991; Marubini and Davies 1996; Ferrier-Pagès et al. 2000*a*) from observations of skeletal length or weight over a relatively long interval (>30 d), while some others have suggested positive effects (Atkinson et al. 1995; Steven and Broadbent 1997). Because skeletal density is not invariable, and thus its extension rate may not always reflect calcium carbonate ($CaCO_3$) precipitation rates (Dodge and Brass 1984), it is necessary to directly measure calcification rates in order to estimate actual C fixation amount as $CaCO_3$. Moreover,

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measurements of calcification rates for corals that have different photosynthetic activity will clarify the relationship between photosynthesis and calcification, which is still a matter for discussion (Gattuso et al. 1999)

Because most of the previous studies set nutrient concentration at considerably high levels (e.g., NO_3^-: 10–50 μmol L^{-1}) compared to in situ levels (usually, <1 μmol L^{-1}), the present study regarded moderate nutrient loading (NO_3^-: <5 μmol L^{-1}, PO_4^{3-}: <0.3 μmol L^{-1}), which can sometimes be seen in coastal areas affected by river input and groundwater discharge (*see* below). The purpose of this study was to investigate those nutrient effects on the balance between organic tissue production and calcification. The nutrient enrichment clearly resulted in increase in tissue biomass and Chl *a* concentration per unit surface area of the coral. Using these coral branches, net C fixation rates into organic tissue and carbonate skeleton were measured in normal nutrient-depleted seawater with a ^{13}C labeling technique. The technique required only a few days to determine the rates and clarified how each C fixation rate is affected by the level of algal Chl *a* concentration.

Material and methods

Coral collection and incubation under nutrient enrichment—The experiment was performed using zooxanthellate corals *Acropora pulchra*, which were collected on the reef flat of Shiraho Reef in Ishigaki Island (24°21–31′N, 124°4–16′E), Japan, in July 2005. It has been reported that groundwater around the coastal area of the Shiraho Reef perennially contains anthropogenic N and that dissolved inorganic nitrogen (DIN) concentration of seawater near the shoreline of the reef is significantly influenced by the groundwater discharge (3.3–230 μmol L^{-1} NO_3^-; Umezawa et al. 2002). Due to rapid dilution of the lagoon water with the offshore waters, the majority of the lagoons had only slightly higher NO_3^- (0.2–1.5 μmol L^{-1}), NH_4^+ (0.2–1.0 μmol L^{-1}), and PO_4^{3-} (0.02–0.08 μmol L^{-1}) values than those in the offshore waters (NO_3^-: <0.2 μmol L^{-1}, NH_4^+: <0.2 μmol L^{-1}, PO_4^{3-}: <0.02 μmol L^{-1}; Miyajima et al. in press), but more N input into the reef would be a concern in the future. Coral branches of ~6 cm in diameter were collected ~500-m offshore, where the depth is ~0.5 m at low tide. Within 1 h after the collection, corals were transferred to an outdoor aquarium, where seawater that contained nutrients (NO_3^-: 2.1 μmol L^{-1}, NO_2^-: 0.06 μmol L^{-1}, NH_4^+: 0.03 μmol L^{-1}, PO_4^{3-}: 0.09 μmol L^{-1}, on average) was continuously supplied. Each coral was suspended using nylon line. NO_3^- and PO_4^{3-} were additionally supplied once a day to achieve a final concentration of 5 μmol L^{-1} and 0.3 μmol L^{-1}, respectively. After the addition of NO_3^- and PO_4^{3-}, the aquarium was kept closed for a few hours to prevent rapid dilution of the nutrients, during which the seawater was agitated with magnetic stirrers.

Corals were collected from the reef at three separate times: One group (n = 8) was sampled 10 d before the following experiment of ^{13}C incorporation, another (n = 9) was 5 d before, and the last group (n = 12) was just sampled 15 h before and was not affected by the extra NO_3^- and PO_4^{3-} addition. Samples for nutrient concentrations were taken in 10 mL acrylic tubes before and after the extra NO_3^- and PO_4^{3-} addition, and stored at −20°C until analysis.

The ^{13}C incorporation experiment—After the nutrient enrichment treatment, three groups of the collected corals were incubated for 4 d with ^{13}C-labeled dissolved inorganic C (DIC). Seawater used during the period contained low nutrients (NO_3^-: 0.11 μmol L^{-1}, NO_2^-: 0.03 μmol L^{-1}, NH_4^+: 0.05 μmol L^{-1}, PO_4^{3-}: 0.03 μmol L^{-1}, average of the 4 d) to minimize changes in coral and algal biomass parameters such as Chl *a* and to measure photosynthetic and calcification rates under the same nutrient condition for the all corals. The seawater was gently stirred with magnetic stirrers and exchanged twice a day (10:00 and 17:00 h). $NaH^{13}CO_3$ was added after each water exchange to get 0.1 mmol L^{-1} excess ^{13}C (~5 atom% ^{13}C). Temperature of the water was controlled by flowing seawater outside the aquarium (28.9°C on average during the ^{13}C incorporation). Corals of each group were randomly arranged in the aquarium and systematically moved every day to minimize position effects such as light intensity and water velocity.

Samples for nutrient concentrations, DIC concentrations, and DIC isotope ratios were taken before and after the seawater exchange. Samples for the DIC concentration and its isotopic ratio were taken in 15-mL and 30-mL glass vials, respectively. After they were fixed with saturated $HgCl_2$ to final concentrations of 0.03 and 0.07% (v/v), respectively, they were sealed with a PTFE (polytetrafluoroethylene)-coated butyl rubber septum and an aluminum seal.

After the ^{13}C incorporation for 4 d, each incubated coral was divided into two tips. One of them was put in 15 mL of methanol to extract whole Chl *a* from the tip. Another part was processed according to Tanaka et al. (2006*a*) in order to separate coral and algal tissue from the skeleton. Because all the tissue was not separated from the skeleton with this procedure, Chl *a* was also extracted from the obtained algal tissue to determine at what ratio the tissue was collected from the skeleton.

Analysis

Dissolved inorganic nitrogen (DIN: NO_3^-, NO_2^-, NH_4^+) and phosphorus (DIP: PO_4^{3-}) were quantified by using a nutrient analyzer AACS-III (BRAN+LUEBBE; detection limit: <0.01 μmol L^{-1}). DIC concentration was measured with a Shimadzu TOC 5000 instrument, and its isotopic ratio was determined with GC-IRMS (gas chromatography–isotope ratio mass spectrometer; Agilent Technology GC-6890 + Finnigan DELTA plus XP; Miyajima et al. 1995). The gas chromatograph was equipped with a capillary column J&W GS-GASPRO (30 m length, 320 μm inner diameter), and oven temperature was kept at 70°C.

Coral and algal tissue suspension was infiltrated into a precombusted glass-fiber filter (Whatman GF/D, 10 mm in diameter). The filters were dried on a hot plate at 80°C and treated with a vapor of 12 mol L^{-1} HCl for 12 h at room temperature to remove inorganic C. After evaporat-

Table 1. Chl *a* concentration and tissue biomass for the coral and zooxanthellae affected by nutrient enrichment for each scheduled period. Data are average ± standard error (SE); n = 12, 9, and 8 for 0, 5, and 10 d enrichment, respectively.

Enriched period (d)	Chl *a* (μg cm^{-2})	Coral (μmol cm^{-2})			Zooxanthellae (μmol cm^{-2})			Zooxanthellae : coral (%)	
		C	N	C : N	C	N	C : N	C	N
0	2.6±0.2	343±29	46±4	7.4±0.1	26±2	3.5±0.4	7.5±0.3	7.5±1.0	7.6±1.1
5	5.2±0.1	495±40	63±6	7.9±0.1	38±3	4.4±0.4	8.8±0.3	7.7±0.9	7.0±0.9
10	6.9±0.3	588±56	72±7	8.1±0.1	52±5	6.0±0.6	8.7±0.2	8.8±1.2	8.3±1.2

ing extra HCl on the hot plate under vacuum, the filters were dried again in an oven at 50°C for a few hours. Amounts and isotope enrichments of C and N on the filter were measured by combination of CHN analyzer (Fisons; NA-1500) and IRMS connected via Conflo-III interface (Thermo-electron Co. Ltd.). The sum of the coral and algal parts was used to calculate net C fixation rates by photosynthesis.

To quantify the calcified ^{13}C, one part of the incubated coral, from which Chl *a* had already been extracted with methanol, was dried in an oven at 50°C and crushed into a fine powder with a grinder (Osaka Chemical Co.; WB-1). About 20 mg of the powder and 24 mL of distilled water were sealed in a glass vial (30 mL) with a butyl rubber septum and aluminum seal. One milliliter of 1 mol L^{-1} HCl was then injected with a polypropylene syringe to dissolve $CaCO_3$ into CO_2 and get CO_2 gas into the headspace of the vial. The ^{13}C enrichment of CO_2 in the headspace was measured by GC-IRMS in the same way as DIC isotopic ratios (Miyajima et al. 1995).

Calculations

Bulk organic C in coral and algal tissue per unit surface area (T; μmol cm^{-2}) was determined from partially separated tissue C (T_p; μmol) and the ratio (R) of Chl *a* was extracted from the obtained algal tissue to the total Chl *a* of the branch, such that

$$T = T_p R^{-1} A^{-1} \quad (1)$$

where A (cm^{-2}) is a skeletal surface area determined by aluminum foil method (Marsh 1970). Bulk organic N was also calculated in the same way.

Skeletal C (S; g) in $CaCO_3$ for each coral tip, from which organic tissue was partially separated, was calculated as follows:

$$S = W - TA(1-R) \times 12.01 \times 10^{-6} \quad (2)$$

where W (g) is a coral tip weight still containing a part of organic tissue. Newly incorporated C (C_{new}; μmol) in organic tissue and carbonate skeleton during the ^{13}C labeling period was calculated using C_{new} = (excess ^{13}C amount of the sample) (average excess atom% DI^{13}C in seawater × 0.01)$^{-1}$, excess ^{13}C amount of the sample = (bulk organic C) × (excess atom% ^{13}C of the sample), and excess atom% ^{13}C of the sample = (atom% of the sample) − (1.108% as a natural ratio). Net photosynthesis and calcification rates were normalized by dividing C_{new} by the labeling period of 4 d and surface area of the coral tips.

Results

Chl a and tissue biomass—The nutrient condition in this study (NO_3^-: <5 μmol L^{-1}, PO_4^{3-}: <0.3 μmol L^{-1}) increased Chl *a* concentration per unit surface area of the coral by a factor of 2.0 for 5 d and 2.6 for 10 d on average (Table 1). The lower increase in rate from day 5 to 10 indicates that Chl *a* content per unit area gradually approached saturation state.

Both coral and zooxanthellate biomass also increased with nutrient enrichment days. Algal C and N biomass increased 2.0-fold and 1.7-fold for 10 d, respectively (Table 1). Thus, Chl *a* : C and Chl *a* : N ratios of the zooxanthellae increased 1.3-fold and 1.5-fold during the period, respectively.

Host C and N biomass increased 1.6-fold and 1.7-fold for 10 d, respectively (Table 1). Biomass ratios of the zooxanthellae to the host coral were not significantly affected for both C and N even after 10 d (Student's *t*-test, p = 0.1 and 0.2, respectively).

Photosynthesis and calcification rates—During the ^{13}C incorporation experiment, DIC concentration of newly exchanged seawater was 1.70 ± 0.02 mmol L^{-1} (average ± standard deviation [SD]), and it decreased to 1.48 ± 0.02 mmol L^{-1} at 17:00 h and to 1.61 ± 0.03 mmol L^{-1} at 10:00 h. Relatively low DIC concentration of the seawater compared to usual ocean concentrations may have been because it was taken from the inner reef during the evening, when DIC concentration decreases due to photosynthesis and calcification during the daytime (Hata et al. 2002).

The rate of net organic C production by the zooxanthellae increased 2.1-fold for the corals that had 5 d nutrient enrichment and 2.8-fold for those at 10 d (Table 2). The increase rate was similar to that of Chl *a* concentration per unit surface area, meaning that the net production per Chl *a* (assimilation number) was almost constant (average: 4.0 μmol C [μg Chl *a*]$^{-1}$ d^{-1}). There was a clear correlation between the Chl *a* concentration and the net photosynthetic rate (r^2 = 0.75, $p < 0.0001$; Fig. 1).

The calcification rate also showed a positive correlation with the Chl *a* (Fig. 1), but the increase in ratio was significantly lower (1.3-fold for the 10-d-enriched corals; Table 2) than that of photosynthesis (2.8-fold), and the correlation coefficient was low (r^2 = 0.18; Fig. 1). Because both of the net photosynthesis and calcification rates were

Table 2. Photosynthetic C fixation rate (P) (μmol C cm^{-2} d^{-1}) and calcification rate (C) (μmol C cm^{-2} d^{-1}) calculated from ^{13}C tracer accumulation. Tissue C : skeletal C is a structural ratio of tissue organic C to skeletal C forming $CaCO_3$ of each coral branch. Extra organic C (μmol C cm^{-2} d^{-1}) was calculated from the difference between the fixation ratio (P : C) and the structural ratio. Data are average ± standard error (SE); n = 12, 9, and 8 for 0, 5, and 10 d enrichment, respectively.

Enriched period (d)	Chl a (μg cm^{-2})	P	C	P : C	Tissue C : skeletal C	Extra organic C
0	2.6±0.2	10.1±0.9	13.3±0.6	0.76±0.06	0.31±0.03	6.0±1.1
5	5.2±0.1	21.0±1.7	12.8±1.1	1.69±0.14	0.49±0.06	15±3
10	6.9±0.3	28.3±2.2	16.8±0.7	1.73±0.19	0.75±0.06	16±4

positively correlated with Chl a, the relationship between photosynthesis and calcification also showed a significant positive correlation (p = 0.01; Fig. 2).

The C fixation ratio of the net photosynthesis to the calcification was 0.76 ± 0.06 for the nutrient-untreated corals (Table 2; Fig. 3). On the other hand, the structural ratio of tissue organic C to skeletal inorganic C was 0.31 ± 0.03 for the same corals (Table 2; Fig. 3). Supposing that the fixation ratio gradually approaches the structural ratio, extra organic C was calculated to be 6.0 μmol C cm^{-2} d^{-1} ($10.1 \times [1 - 0.31 \times 0.76^{-1}]$). For the 10-d nutrient-treated corals, the C fixation ratio was 1.73 ± 0.19 and the structural ratio was 0.75 ± 0.06 (Table 2; Fig. 3). The extra organic C was calculated to be 16 μmol C cm^{-2} d^{-1} for these corals (Table 2).

Discussion

Algal C and N biomass increased with the number of nutrient-enriched days (Table 1), and the ratio of Chl a concentration to the algal biomass also increased during the first 5 d. These results suggest an increase in algal population density per unit area and/or an increase in Chl a content per algal cell. Both of them can be considered, because similar observations have been found in previous studies with enrichment of NH_4^+ (e.g., Hoegh-Guldberg and Smith 1989; Stambler et al. 1991; Muller-Parker et al. 1994) and NO_3^- (Marubini and Davies 1996). Hoegh-Guldberg and Smith (1989) observed with *Stylophora pistillata* and *Seriatopora hystrix* that the mean cell diameter of the zooxanthellae was unaffected by elevated NH_4^+ (10–40 μmol L^{-1} for 3 weeks). Snidvongs and Kinzie (1994) also reported that C contents per zooxanthellate cell of *Pocillopora damicornis* were not influenced by 15 μmol L^{-1} NH_4^+ enrichment for 8 weeks. These observations suggest that nutrient enrichment increases the population density of zooxanthellae, but the cell size is kept almost constant.

Not only the abundance of zooxanthellae, but host coral biomass also increased with the nutrient treatment, maintaining a relatively constant algal : coral biomass ratio (Table 1). This implies that host tissue growth is stimulated by NO_3^- (Tanaka et al. 2006*a*), as well as NH_4^+ (Muller-Parker et al. 1994). Because enzymes for NO_3^- assimilation, i.e., NO_3^- and NO_2^- reductases, have never been found in host corals, the enhanced host tissue growth could be due to translocation of N-rich compounds from the zooxanthellae (Tanaka et al. 2006*a*). Grover et al. (2003) assessed the uptake rates of NO_3^- by *Stylophora pistillata* using the ^{15}N technique, and found that the ^{15}N enrichment of the algal fraction was up to 12 times greater than that of the host, which suggests that the zooxanthellae are the primary site of NO_3^- incorporation.

Yet, considering that NO_3^- has to cross at least two animal membranes to reach the zooxanthellae, Grover et al. (2003) implied that the host has a role in NO_3^- assimilation by the zooxanthellae. Some other works have also suggested that the host is primarily involved in N uptake (Miller and

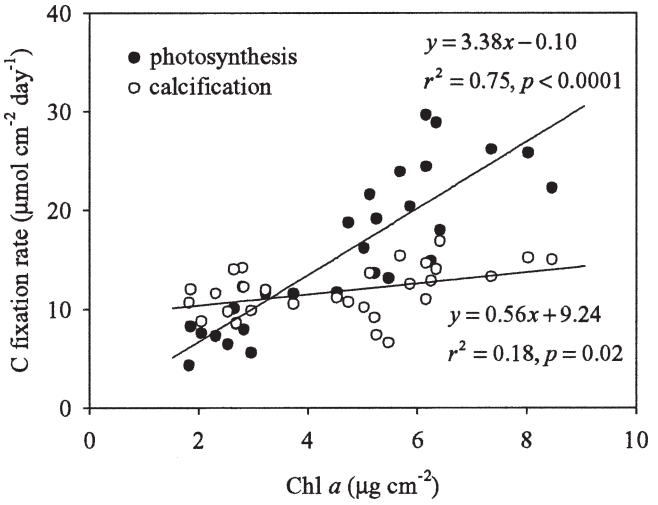

Fig. 1. Relationship between Chl a (μg cm^{-2}) and C fixation rates (μmol cm^{-2} d^{-1}) of net photosynthesis and calcification.

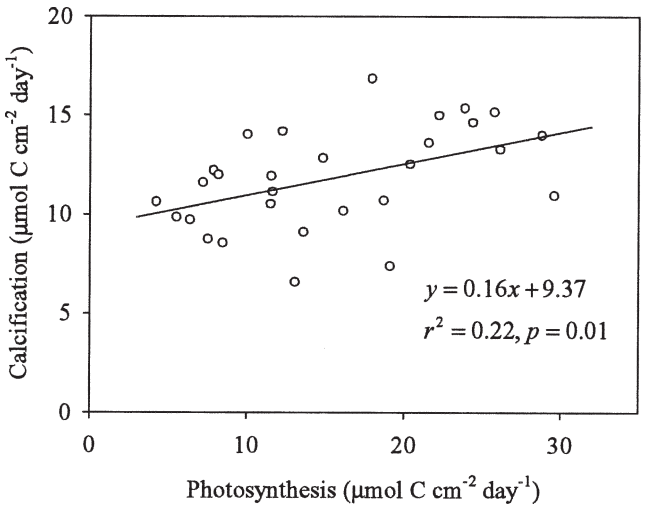

Fig. 2. Relationship between net photosynthesis and calcification.

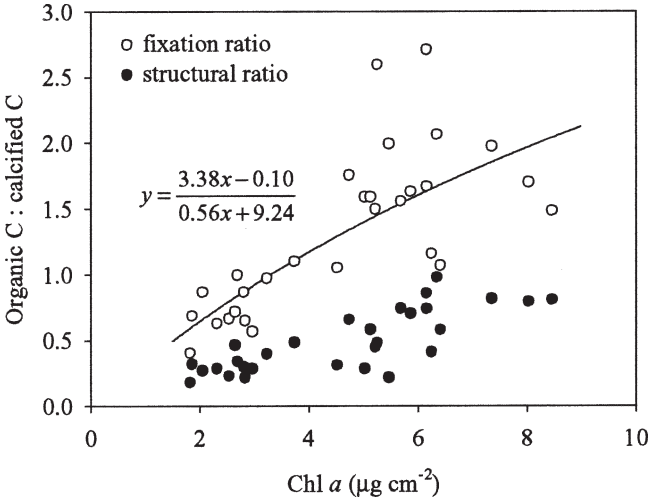

Fig. 3. Differences in organic C : skeletal C ratios between the measured C fixation ratios and the structural ratios of each coral branch, depending on the Chl *a* concentration of zooxanthellae (μg cm^{-2}). The fitting curve for the fixation ratio is calculated from the linear regressions in Fig. 1.

Yellowlees 1989; Wang and Douglas 1998). Miller and Yellowlees (1989) proposed two possibilities for the host role: (1) that NO_3^- transport across the animal membrane is mediated by a nonspecific carrier with a physiological function that is the transport of another species and (2) that the host synthesizes a specific NO_3^- carrier for the sole benefit of the zooxanthellae. On the other hand, they also implied that the ability of coral colonies to utilize ambient NO_3^- may depend on the environmental nutrient status: In corals that reside in relatively high-nutrient environments, the NO_3^- uptake system should be repressed due to readily available reduced N, such as NH_4^+ and amino acids. Considering that coral colonies used in the present study actively took up NO_3^- in their tissue, they might have been originally in a nutrient-limited environment. Translocation of organic N from zooxanthellae to the host may also depend on the host nutrient condition.

The ^{13}C incorporation experiment revealed that the net photosynthetic rate increased nearly in direct proportion to the Chl *a* concentration per unit area (Fig. 1; Table 2). Though some studies have suggested DIC limitation of zooxanthellate photosynthesis due to a diffusional boundary layer (Lesser et al. 1994), our results suggested that the algal photosynthesis was not limited by DIC in the seawater, at least within the range of the Chl *a* observed in this experiment (Burris et al. 1983). Whether the DIC limitation effect occurs or not probably depends on water velocity, which affects boundary layer thickness, and on the individual coral species, which could have different Chl *a* concentrations and metabolic rates.

The increase in rate of calcification was less than half of that of the net photosynthesis (Fig. 1; Table 2). It has long been thought that photosynthesis stimulates calcification, as observed by calcification rates that are higher in light than in dark (e.g., Kawaguti and Sakumoto 1948), although the interactions between the two reactions are still matters of continuous controversy. Gattuso et al. (1999) reviewed the literature and found that the average ratio of light : dark calcification rates was 3.0, indicating that photosynthesis considerably affects calcification rates. One of the well-known mechanisms to explain higher calcification rates in light (light-enhanced calcification) begins with photosynthetic CO_2 uptake by zooxanthellae (Eq. 3), which increases pH in seawater by a shift in carbonate equilibrium (Eq. 4). This pH shift increases carbonate ion concentration (Eq. 5) and consequently stimulates $CaCO_3$ precipitation according to Eq. 6 (Goreau 1959).

$$CO_2 + H_2O \rightarrow CH_2O + O_2 \quad (3)$$

$$HCO_3^- \rightarrow CO_2 + OH^- \quad (4)$$

$$HCO_3^- + OH^- \rightarrow CO_3^{2-} + H_2O \quad (5)$$

$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3 \quad (6)$$

The hypothesis of McConnaughey and Whelan (1997) brought up the importance of H^+ produced by calcification (Eq. 7), and they suggested that calcification may stimulate photosynthesis (Eq. 7 $\rightarrow$ 4 $\rightarrow$ 3).

$$Ca^{2+} + HCO_3^- \rightarrow CaCO_3 + H^+ \quad (7)$$

However, this model was recently contradicted by Gattuso et al. (2000), who demonstrated that light-enhanced calcification can be inhibited without influencing photosynthesis. Another most recent hypothesis for the light-enhanced calcification is based on the titration of H^+ produced by calcification (Eq. 7) with the OH^- produced by photosynthesis (Furla et al., 1998, 2000).

In those previous studies, however, nutrient effects were disregarded. Nutrient incorporation generally enhances algal photosynthetic activity, and, accordingly, gross and net primary production (Dubinsky et al. 1990; Marubini and Davies 1996; Ferrier-Pagès et al. 2000*a*) as also found in the present study. Here, attention was paid to how calcification was affected by the increased photosynthetic DIC uptake due to nutrient incorporation. As a result, the relationship between net photosynthesis and calcification showed statistically significant correlation ($p = 0.01$; Fig. 2), but the slope of 0.16 suggests that the calcification was not considerably stimulated by the enhanced photosynthetic activity. In other words, the light-enhanced calcification was almost at a maximum level, and other factors such as organic matrix synthesis (*see* below) limit the calcification. Even moderate nutrient loading for 10 d in the present study disrupted the balance between photosynthesis and calcification, implying that the skeletal extension could not keep up with the tissue growth at least in a short time interval.

Even though the increase in rate of calcification was significantly lower than that of net photosynthesis, this study is one of the few reports to suggest a positive effect of nutrients on coral calcification (Meyer and Schultz 1985; Atkinson et al. 1995; Steven and Broadbent 1997). Some possibilities can be suggested for the increased calcification with the nutrient-enhanced photosynthesis. First, the light-enhanced calcification was directly raised by the increased algal photosynthesis. Algal photosynthesis enhanced by

nutrient incorporation would produce more OH^- (Eqs. 3 and 4) and increase CO_3^{2-} (Eq. 7), taking into account the hypothesis of Goreau (1959). Otherwise, from the perspective of the titration hypothesis, enhanced photosynthesis would alkalize coral coelenteric space more strongly (Eqs. 3 and 4) and may facilitate diffusion of H^+ produced by the $CaCO_3$ precipitation (Eq. 7), which results in enhancement of the calcification (Furla et al. 1998).

Second, the nutrient enrichment enhanced synthesis of an organic matrix, which is a prerequisite step for the formation and growth of coral skeleton. It has been reported that coral skeleton contains organic matter such as mucopolysaccharides (Goreau 1959), proteins (e.g., Allemand et al. 1998), calcium-binding phospholipids (Isa and Okazaki 1987), and glycoproteins (Constantz and Weiner, 1988). Allemand et al. (1998) demonstrated that inhibition of protein synthesis reduced Ca deposition rate simultaneously, suggesting that organic matrix biosynthesis, rather than calcium deposition, may be a limiting factor controlling the coral skeletogenesis. In the present study, the nutrient enrichment could have increased amino acid synthesis by the zooxanthellae. This higher amount of amino acids would have been translocated to the host and would have increased host biomass (Table 1). Although the C : N ratio of the host did not significantly change, a protein pool for synthesis of the organic matrix might have been enlarged with the nutrient pretreatment. Several authors have shown that photosynthetic products of zooxanthellae may be used as precursors for the organic matrix (e.g., Young et al. 1971).

Third possibility is that the increased supply of metabolic CO_2 in the calicoblastic cell enhanced the calcification rate. Furla et al. (2000) showed that metabolic CO_2 would be the main source (70–75%) of total C for calcification. It has also been shown that respiration rates can be increased by higher nutrient conditions and subsequent coral biomass increases (Marubini and Davies 1996; Ferrier-Pagès et al. 2000*a*), as observed in the present study (Table 1). The nutrient input in this study might have stimulated calcification through increased tissue biomass and supply of metabolic CO_2 (Furla et al. 2000).

Unlike the present results, many others have conversely reported negative effects of nutrient enrichment on calcification (Stambler et al. 1991; Marubini and Davies 1996; Ferrier-Pagès et al. 2000*a*). One well-known hypothesis to explain the decreased calcification is that photosynthesis and calcification may compete for the same saturated DIC pool (Stambler et al. 1991). Photosynthetic activity stimulated by nutrient enrichment may draw down the internal DIC pool for calcification. Langdon and Atkinson (2005) measured photosynthesis and calcification rates in low-nutrient seawater after exposing corals to very high-nutrient conditions (NH_4^+: 109 μmol L^{-1}, PO_4^{3-}: 13 μmol L^{-1}) for 4 h. They observed that the increase in photosynthesis was almost mole for mole with the decline in calcification, suggesting that under saturation of the internal nutrient pool, the two C fixation processes compete for the same DIC pool.

However, at least under the condition of the present study, both photosynthesis and calcification more or less increased, suggesting that the internal DIC pool was not saturated and that competition for the DIC pool might not have occurred. Whether or not the internal DIC pool is saturated and competition for the pool occurs might depend on several conditions, such as nutrient levels, exposure period to the nutrients, and subsequent change in Chl *a* concentration. In most observations that have reported significant growth-rate reduction (Stambler et al. 1991; Marubini and Thake 1999; Ferrier-Pagès et al. 2000*a*), DIN concentration was set at >15 μmol L^{-1}, except for Marubini and Davies (1996). This very high level of DIN compared to in situ concentrations could cause saturation of the internal DIC pools (Langdon and Atkinson 2005) and subsequent competition for the limited DIC supply, which might have reduced skeletal growth rates.

In the study of Marubini and Davies (1996), a significant decrease in calcification rates of *Porites porites* and *Montastrea annularis* was observed by 30% to 60%, even using a relatively low range of NO_3^- concentrations (1, 5, and 20 μmol L^{-1}). They also adopted the hypothesis of DIC competition between photosynthesis and calcification, although the hypothesis did not fit the case of the corals under 1 μmol L^{-1} NO_3^- condition, where calcification was reduced without any change in photosynthesis. The different result in calcification from the present study might have derived from nutrient availability during measurement of calcification: While Marubini and Davies (1996) measured the calcification rate under the each NO_3^- concentration, calcification in the present study was measured under the nutrient-depleted condition for the all corals in order to focus on the direct effect of photosynthetic DIC uptake rates on the calcification.

With nutrients, zooxanthellae use more of their photosynthetic products for their own growth, and, consequently, translocation of the algal photosynthates to the host could be decreased (Dubinsky et al. 1990). In Marubini and Davies (1996), the calcification rate under relatively low NO_3^- concentrations, such as 1 μmol L^{-1}, might have been reduced by the decreased translocation of organic matter (Stambler et al. 1991), a part of which may have been used for synthesis of organic matrix as mentioned above (Allemand et al. 1998). In the present study, the reduction of translocation during measurement of photosynthesis and calcification could have not occurred because nutrients were depleted during the period. Therefore, calcification rates might decrease by nutrient enrichment according to the processes of not only DIC competition between photosynthesis and calcification but also reduced translocation of photosynthetic products to the host. This is supported by the fact that the DIC competition hypothesis does not fit the case of the 1 μmol L^{-1} NO_3^- condition in Marubini and Davies (1996).

Some authors have also observed negative effects of nutrient enrichment on coral growth from in situ investigation (Walker and Ormond 1982; Tomascik and Sander 1985). Although all of the studies also found a negative effect, it could be difficult to evaluate nutrient effects alone, because many other environmental factors, such as temperature, light intensity, and water movement, simultaneously have impacts on corals in actual reefs. Tomascik and Sander (1985) monitored those environmental variables and coral growth rates at several locations that

were under different degrees of environmental stress caused by increased eutrophication. Though both concentrations of inorganic N (<0.5 μmol L^{-1}) and P (<0.01 μmol L^{-1}) were negatively correlated with the growth rate, they concluded that the nutrients had little or no direct causal effect on growth themselves and that concentration of suspended particulate matter was the best univariate estimator of coral growth rates.

The measured C fixation ratio of net photosynthesis to calcification was 0.76 for the corals untreated with nutrients (Table 2). This corresponds well to the average ratio, 0.77, of previous studies (reviewed by Gattuso et al. 1999). On the other hand, structural ratios of coral plus algal C biomass to skeletal C for each coral branch were 0.31 on average, which were significantly (60%) lower than the measured fixation ratio (Table 2). Assuming that the structural ratio would keep constant under the incubation condition, the extra organic C (6.0 μmol C cm^{-2} d^{-1}) should be gradually consumed for respiration. Most of newly produced respiratory substrates must be lost from the tissue within 24 h after synthesis, but some of them could be gradually consumed over a longer period (Tanaka et al. 2006*a*). Another possibility for the extra organic production is organic matter release to the ambient seawater. It is well known that corals release some parts of photosynthetically fixed C as dissolved and particulate organic matter (e.g., Crossland 1987; Ferrier-Pagès et al. 1998; Wild et al. 2004).

For the nutrient-enriched corals for 10 d, the C fixation ratio increased 2.3-fold (Table 2; P : C = 1.73 ± 0.19), and the structural ratio was also raised comparably (2.4-fold). Assuming that the structural ratio was already at steady state, the extra organic C production mentioned above was equivalent to 16 μmol C cm^{-2} d^{-1}. In this case, even considering that the enlarged tissue biomass (Table 1) leads to a respiration increase roughly in proportion to the biomass, the extra primary production and subsequent organic matter release might become greater with Chl *a*. Whether or not organic C release from coral colonies is stimulated by nutrient enrichment remains to be resolved (Ferrier-Pagès et al. 1998; Tanaka et al. 2006*b*).

Given longer time, the structural ratio might approach the observed C fixation ratio, even for the corals that have a high Chl *a* concentration. In this case, the estimated extra organic C (16 μmol C cm^{-2} d^{-1}) could be more or less overestimated. However, a question that arises here is what range of tissue : skeleton ratios is appropriate or possible for the coral growth. If photosynthetic products were stored in the tissue at the observed fixation ratio, tissue : skeleton ratios would gradually increase under the high Chl *a* concentration, and at some future point, the carbonate skeleton might brim over with the excessively produced organic matter. To examine the problem, a longer-term experiment is required.

In summary, nutrient levels in this study (<5 μmol L^{-1} NO_3^- and <0.3 μmol L^{-1} PO_4^{3-}) increased zooxanthellate Chl *a* concentration per unit surface area, and photosynthetic C fixation rates were enhanced in proportion to the Chl *a*. Tissue biomass of both the host coral and the zooxanthellae was consequently doubled for 10 d of nutrient enrichment. In contrast, calcification was only left behind with respect to C fixation rates. Unless carbonate skeletal density is changed, the skeletal extension might not keep up with the tissue growth.

Although the increased ratio of calcification was less than that of the net photosynthesis, the present results suggest that competition for the same internal DIC pool between photosynthesis and calcification does not always occur, at least within the range of Chl *a* in this study. Both hypotheses, i.e., the DIC competition and the reduction of organic matter translocation from zooxanthellae to the host, might be involved in decreased calcification found in the previous studies.

As algal Chl *a* concentration increases, more produced organic C will be stored for tissue growth and/or excreted as organic matter into the ambient seawater. In the former case, it should be investigated how this imbalance between the two C fixation rates affects longer-term coral growth. It may cause excess and even pathological tissue hypertrophy and finally lead to competition for the internal DIC pool between photosynthesis and calcification as implied in other previous studies (Stambler et al. 1991; Marubini and Davies 1996). In the latter case, the excreted organic matter could be utilized by microorganisms, and therefore change organic C flux from the coral colony to detritus food webs in the reef water. Several studies have focused on this phenomenon (Ferrier-Pagès et al. 2000*b*; Wild et al. 2004). Because zooxanthellae in hermatypic corals are one of the major primary producers in coral reefs, net CO_2 fixation of a whole reef would be affected by the rate at which released organic matter is mineralized to CO_2.

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